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            Markers of Breast Cancer

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14. ABSTRACT

Experimental data suggest that a high ratio of omega-6 polyunsaturated fatty acids (PUFAs) to omega-3 PUFAs promotes breast cancer. Although the exact mechanism by which this occurs is unknown, it is suggested that the 6:3 PUFA ratio influences breast cancer development by affecting eicosanoid synthesis. Specifically, when omega-3 PUFAs displace omega-6 PUFAs, prostaglandin E2 synthesis (PGE2) is reduced resulting in decreased aromatase activity and suppression of estrogen synthesis. Whether the reduction in PGE2 substantially affects circulating estradiol levels or localized estradiol production in breast tissue is unknown. Therefore, we sought to determine the relationship between the erythrocyte 6:3 PUFA ratio and serum estradiol and percent breast density, two breast cancer risk factors. We hypothesized that the 6:3 PUFA ratio would be positively associated with both risk factors. Because nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit PGE2 formation, we further hypothesized that the association between the 6:3 PUFA ratio and estradiol and breast density would differ by NSAID use. Analyses revealed that among non-users of NSAIDs, the 6:3 PUFA ratio was positively and significantly correlated with estradiol (r=0.27;p=0.0019). However, this significant finding was not observed among NSAID users (r=0.14;p=0.1345). Analyses assessing the relationship between the 6:3 PUFA ratio and breast density are underway.

15. SUBJECT TERMS

omega-3 fatty acids, omega-6 fatty acids, estradiol breast density

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1 Introduction

Extensive experimental evidence has shown that intake of omega-6 polyunsaturated fatty acids (PUFAs) promotes breast cancer (1), while consumption of omega-3 PUFAs inhibits this disease (2). Furthermore, it appears that the cancer promoting activity of the omega-6 fatty acids is abrogated by the competitive inhibition of omega-3 fatty acids (3, 4). Although the mechanism by which the 6:3 PUFA ratio may promote breast cancer is unknown, it is suggested that a high 6:3 PUFA ratio may influence breast cancer risk by increasing prostaglandin E2 (PGE2) synthesis thereby inducing estrogen production, via upregulation of aromatase. Whether the increase in PGE2 can cause substantial affects on circulating estradiol levels or localized estradiol levels in breast tissue remains undetermined.

Therefore, utilizing fatty acids in erythrocytes as a biomarker of recent dietary intake, we sought to determine if the 6:3 PUFA ratio was related to two postmenopausal breast cancer risk factors, circulating estradiol levels and percent mammographic breast density. We hypothesized that the 6:3 PUFA ratio would be positively associated with both serum estradiol concentration and percent breast density in cancer-free postmenopausal women not taking exogenous hormones. Moreover, because nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit PGE2 formation, we further hypothesized that the association between the 6:3 PUFA ratio and estradiol and breast density would differ between women using and not using NSAIDs.

2 Body

The primary study objectives, to determine the relationship between the 6:3 PUFA ratio and serum estradiol levels and percent mammographic breast density, will be assessed by undergoing an ancillary study, within an existing case-control study.

Parent study overview: The Mammograms and Masses Study (MAMS) is a case-control study of estrogen metabolites, mammographic density and breast cancer risk. 869 cancer-free women and 264 recently diagnosed breast cancer cases were recruited into the MAMS through the Magee Womens Hospital Mammographic Screening and Diagnostic Imaging Program in the greater Pittsburgh area (Pennsylvania, USA) between 2001 and 2005. Women who were 18 years or older and reported no previous personal history of cancer, with the exception of nonmelanoma skin cancer, were eligible for study enrollment. Participants in the MAMS include: 1) women recruited from the Magee-Womens Surgical Clinic for an initial evaluation after newly diagnosed breast cancer; 2) women who were undergoing outpatient needle breast biopsy, but who were not subsequently diagnosed with breast cancer, and; 3) women receiving screening mammography through Magee-Womens Hospital or through Pittsburgh Magee Womancare Centers.

Ancillary study population: To be eligible for our ancillary study, participants were required to be enrolled via mechanism three listed above, cancer-free, postmenopausal, and not using exogenous hormones. 260 women were found eligible for our ancillary study. These participants have available anthropometric and demographic data and all 260 are included in the analysis assessing the relationship between the 6:3 PUFA ratio and serum estradiol. However, mammographic density readings were not obtained on all participants; therefore, for the purposes of the breast density endpoint it was necessary to further restrict this population to women with

an available mammogram. 3 of the 260 participants do not have an available mammogram for which a density reading could be obtained.

Erythrocyte fatty acid measurements: Erythrocyte fatty acid levels were identified using gas-liquid chromatography. All samples were delivered to the University of Pittsburgh's Heinz Nutrition Laboratory in the Department of Epidemiology for analysis. Total lipids (500µl of packed red blood cells) were extracted from EDTA-containing blood samples according to the general technique of Bligh and Dyer(5). Identification of fatty acids was by comparison of retention times with those of authentic standards (Sigma). A random subset of 27 samples was analyzed for reproducibility. The coefficients of variation (CV) between runs for individual omega-6 fatty acids and omega-3 fatty acids were all < 10%.

Serum estradiol analysis: Serum samples were shipped to the Royal Marsden Laboratory for analysis. Estradiol levels were measured by radioimmunoassay using a highly specific rabbit antiserum raised against an estradiol-6-carboxymethyloxime-bovine serum albumin conjugate (EIR, Wurenlingen, Switzerland) and Third Generation Estradiol [I125] reagent DSL 39120 (Diagnostic Systems Laboratories Inc., Texas, USA). Duplicate samples were extracted with 2ml of diethyl ether. The extract was dried down and reconstituted in 0.5ml phosphate buffered saline pH 7.3 containing 0.1% gelatine. The reconstituted extract was incubated overnight at 4°C with 0.1ml antiserum diluted 1:4 in assay buffer and 0.1ml Estradiol [I125] reagent diluted 1:4 in assay buffer. After incubation, the bound and free fractions were separated using a solid phase second antibody coated cellulose suspension (Sac-Cel IDS Ltd, Tyne & Wear, U.K.). After centrifugation, the bound fraction was counted in a gamma counter and the amount of estradiol in the samples was determined from a calibration curve. The sensitivity of the assay is defined as 3 pmol/l by calculation from the 95% confidence limits of the zero standard. A random subset of 27 samples was analyzed for reproducibility. The calculated CV between runs was 14.5%.

Mammographic breast density readings: Copies of mammographic films were obtained on all but three participants and sent to an expert reader for determination of percent mammographic breast density. Mammographic measurements were made using a randomly selected craniocaudal view of one breast from each subject. To calculate percent breast density, areas of radiographically dense tissue were outlined with a wax pencil, excluding biopsy scars, Cooper's ligaments, and breast masses. Total breast area and outlined regions were measured using a compensating polar planimeter (LASICO, Los Angeles, CA). Percentage breast density was calculated by dividing the outlined regions by the total breast area. Twenty-one randomly selected mammograms (7 from each tertile of density), were read blindly a second time by the reader. The intraclass correlation coefficient (ICC) for percent breast density was $\rho=0.96$.

Statistical analysis: Preliminary statistical analyses were conducted to assess the relationship between the 6:3 PUFA ratio and circulating estradiol levels. Pearson's correlation coefficients were calculated to examine the magnitude of the relationship between the 6:3 PUFA ratio and natural log-transformed serum estradiol. Partial correlation coefficients were calculated controlling for the effects of age and body mass index (BMI). Because NSAIDs inhibit PGE2 formation, separate correlation analyses were performed for participants reporting NSAID use at time of blood draw and for those not taking these drugs. We formally tested whether NSAID use modified the association between the 6:3 PUFA ratio and serum estradiol by including an

interaction term in an age- and BMI- adjusted general linear model (GLM). Data were analyzed using SAS statistical programs (SAS Institute, Cary, NC). Statistical analyses assessing the relationship between the 6:3 PUFA ratio and breast density are in progress

Results: The sample had a mean±SD serum estradiol concentration of 27.1±25.2 pmol/L, BMI of 29.4±7.1, age of 62.7±8.4 years, and was primarily Caucasian (93.1%). Pearson's correlation coefficients and respective p-values are displayed in **Table 1**. Among all women, the 6:3 PUFA ratio was significantly correlated with serum estradiol levels (r=0.25; p<0.0001) and remained significant after adjustment for age and BMI (r=0.19; p=0.0019). In stratified analyses, the 6:3 PUFA ratio was significantly associated with estradiol among nonusers of NSAIDs (r=0.27; p<0.0019). However, a significant association between the 6:3 PUFA ratio and serum estradiol was not observed among the subgroup taking NSAIDs (r=0.14; p=0.1345). In GLM, we found the interaction between NSAID use and the 6:3 PUFA ratio to be statistically significant (p=0.04). Comparing women with the highest *vs.* lowest tertile 6:3 PUFA ratio, we found estradiol concentration differed by approximately 7.2 pmol/l among NSAID non-users, but by only approximately 2.7 pmol/l among NSAID users (**Figure 1**).

Table 1. Correlations between the 6:3 PUFA ratio and natural log-transformed estradiol levels

	r*	p	r**	p	r***	p
All Women (N=260):	0.25	<0.0001	0.24	0.0001	0.19	0.0019
NSAID Use:						
User (N=125)	0.20	0.0024	0.19	0.0310	0.14	0.1345
Non-user (N=135)	0.30	0.0004	0.30	0.0004	0.27	0.0019

*   Unadjusted
** Partial correlation coefficient adjusted for age
***Partial correlation adjusted for age and body mass index (BMI)

Figure 1. Age- and BMI- adjusted geometric mean serum estradiol concentration (±95% confidence intervals), according to NSAID use and 6:3 PUFA ratio tertile
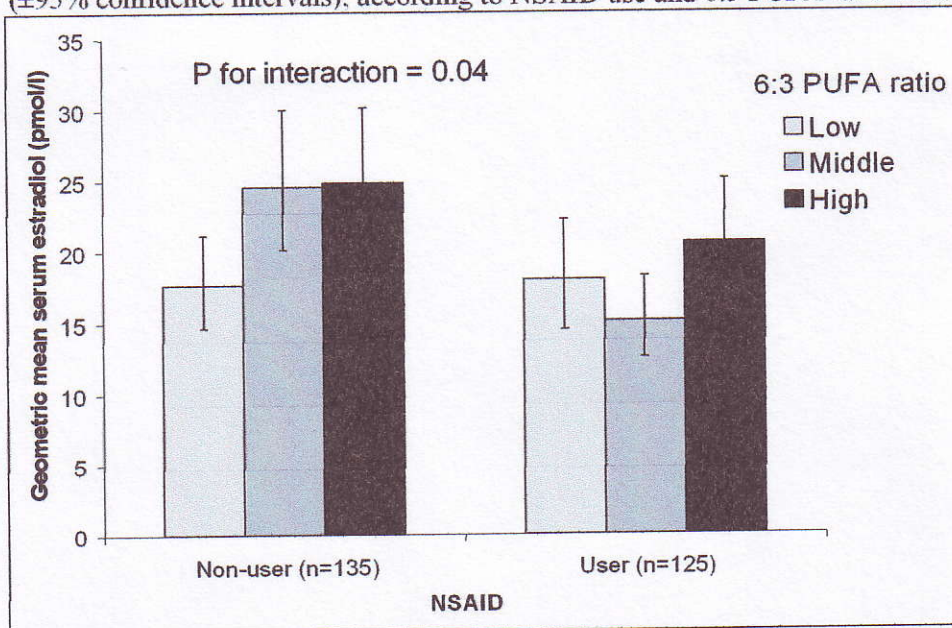
3 Key Research Accomplishments

During the course of the first year, significant research and training accomplishments were made.

Research Accomplishments:
1. Eligible study participants were identified
2. Biological specimens were collected and relabeled with dummy identification numbers for estradiol and fatty acid analysis. 10% of the samples were relabeled as duplicates to validate laboratory results
3. Mammographic films for study participants were collected. Identifying information was removed and the films were shipped for density readings
4. Participants serum samples and 27 duplicate serum samples were shipped to Royal Marsden for estradiol analysis
5. Participants red blood cells and 27 duplicate samples were sent to the Heinz Nutrition Laboratory at the University of Pittsburgh for erythrocyte fatty acid analysis
6. Red blood cell fatty acids, serum estradiol and mammographic density measurements were received and data was entered into the study database
7. Preliminary analyses were conducted on the relationship between the 6:3 PUFA ratio and serum estradiol levels
8. Abstract on the 6:3 PUFA ratio and circulating estradiol levels was accepted for poster presentation at the American Association for Cancer Research (AACR)

Training Accomplishments:
1. Participated and attended linear regression course without benefit of a grade or credit
2. Was trained in two dimensional gel electrophoresis in the laboratory of Dr. Paul Reynolds
3. Successfully passed the comprehensive examination and was admitted to doctoral candidacy
4. Attended the 2006 University of Pittsburgh Cancer Institute Scientific Retreat, Pittsburgh, PA
5. Attended the conference entitled, "Nutrition and quality health among older individuals: what should we recommend?" Pittsburgh, PA
6. Became involved in the PREFER study, which provided training on the design, conduct and statistical analysis of a randomized clinical trial
7. Participated in the University of Pittsburgh Graduate School of Public Health training session on how to write an abstract
8. Attended the American Association for Cancer Research (AACR) Annual Meeting 2007. Los Angeles, CA
9. Awarded the AACR scholar-in-training award, an award given to presenters of highly rated submitted abstracts (Appendix) in all fields of cancer research
10. Abstract was accepted to the University of Pittsburgh Graduate School of Public Health Deans' Day competition and received 2nd place for best overall presentation for my poster on the 6:3 PUFA ratio and postmenopausal estradiol levels

4. Reportable Outcomes

Abstracts:

1. **Hudson A**, Weissfeld J, Modugno F, Wilson J, Evans R, Gierach G, Simpson J, Vogel V. The 6:3 PUFA ratio and serum estradiol in postmenopausal women. American Association for Cancer Research Annual Meeting 2007. Los Angeles, CA. April 14-18, 2007.

5. Conclusion

In conclusion, our preliminary results suggest that the red blood cell 6:3 PUFA ratio is positively correlated with serum estradiol, a causal breast cancer risk factor, among cancer-free postmenopausal women not on hormone therapy. Our findings further indicate that NSAID use attenuates or eliminates the apparent effect of the 6:3 PUFA ratio on estradiol levels. Interventions, such as fish oil supplementation, that reduce the 6:3 PUFA ratio may result in decreased estradiol production and potentially breast cancer risk. However, these interventions may be less effective or ineffective among women who use NSAIDs. To our knowledge, this is the first study to assess the relationship between the 6:3 PUFA ratio and serum estradiol and further research is needed to confirm these findings. Analyses are underway to assess the relationship between the 6:3 PUFA ratio and percent mammographic breast density.

6 References

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2. Rose DP, Connolly JM. Effects of dietary omega-3 fatty acids on human breast cancer growth and metastases in nude mice. J Natl Cancer Inst 1993;85:1743-1747.
3. Karmali RA, Marsh J, Fuchs C. Effect of omega-3 fatty acids on growth of a rat mammary tumor. J Natl Cancer Inst 1984;73:457-461.
4. Fay MP, Freedman LS, Clifford CK, DN M. Effect of different types and amounts of fat on the development of mammary tumors in rodents: a review. Cancer Res 1997;57:3979-3988.
5. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Med Sci 1959;37:911-917.

7 Appendices

1. **Hudson A**, Weissfeld J, Modugno F, Wilson J, Evans R, Gierach G, Simpson J, Vogel V. The 6:3 PUFA ratio and serum estradiol in postmenopausal women. American Association for Cancer Research Annual Meeting 2007. Los Angeles, CA. April 14-18, 2007. (page 9)
2. Curriculum Vitae for Ms. Alana Hudson (pages 11-12)

The 6:3 PUFA ratio and serum estradiol in postmenopausal women

Alana Hudson, Joel Weissfeld, Francesmary Modugno, John Wilson, Rhobert Evans, Gretchen Gierach, Jennifer Simpson, Victor Vogel. University of Pittsburgh, Pittsburgh, PA

Background: Although omega-6 polyunsaturated fatty acids (PUFAs) have been demonstrated to promote breast tumorigenesis and omega-3 PUFAs have been found to prevent breast cancer, the exact mechanisms are unknown. *In vitro* studies suggest that these two families of fatty acids influence breast cancer risk by impacting eicosanoid synthesis. In particular, when omega-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), displace omega-6 arachidonic acid (AA), prostaglandin E2 synthesis (PGE2) is reduced, thus resulting in decreased aromatase activity, and ultimately suppression of estrogen biosynthesis. Based on these laboratory observations, we sought to determine whether the ratio of AA:EPA+DHA in red blood cells (RBC) is positively associated with serum estradiol concentrations in postmenopausal women. **Methods:** Participants in this cross-sectional analysis included 260 cancer-free postmenopausal controls enrolled in the Mammograms and Masses Study (MAMS), a case-control study in Pittsburgh, PA. Only participants not taking hormone therapy, antiestrogens or corticosteroids at blood draw were included in the present analysis. RBC fatty acids were measured by gas chromatography. Estradiol was measured in serum using an indirect radioimmunoassay, and values were logarithmically transformed to obtain normal frequency distributions. Pearson's correlation coefficients were calculated to examine the relationship between the AA:EPA+DHA ratio and serum estradiol. Partial correlation was also performed to control for the effects of age and body mass index (BMI). **Results:** The sample had a mean±SD serum estradiol concentration of 27.1±25.2 pmol/L, BMI of 29.4±7.1, age of 62.7±8.4 years, and was primarily Caucasian (93.1%). Among all 260 participants, the AA:EPA+DHA ratio was positively and significantly correlated with serum estradiol ($r=0.25$; $p<0.0001$) and remained significant after adjustment for age and BMI ($r=0.19$; $p=0.002$). Because anti-inflammatory drugs inhibit AA metabolism and PGE2 formation, separate analyses were performed for participants reporting anti-inflammatory drug use at time of blood draw and for those not taking these drugs (135 nonusers and 125 users). Adjusting for age and BMI, the AA:EPA+DHA ratio was significantly associated with estradiol among nonusers($r=0.27$; $p<0.002$). However, a significant association was not observed among the subgroup taking anti-inflammatory drugs ($r=0.14$; $p=0.13$). **Conclusions:** To our knowledge, this is the first study to report on the association between the AA:EPA+DHA ratio in RBC and serum estradiol in postmenopausal women. Our results suggest that among women not using anti-inflammatory agents, increasing intake of the long chain omega-3 PUFAs, EPA and DHA, and reducing intake of the omega-6 PUFA, AA, may result in decreased estradiol production and potentially breast cancer risk. Further research is needed to confirm these findings.

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PROFESSIONAL EXPERIENCE

Graduate Student Researcher, Graduate School of Public Health

UNIVERSITY OF PITTSBURGH, Pittsburgh, Pennsylvania, 9/2004–4/2006

Responsibilities included assisting with study recruitment and IRB preparation as needed for the Mammographic and Masses Study (MAMS), as well as aided in the preparation of research proposals.

Research Assistant, Graduate School of Public Health

UNIVERSITY OF PITTSBURGH, Pittsburgh, Pennsylvania, 10/2003–8/2004

Involved in the collection of clinical data and entering, cleaning, and verifying all data collected for MAMS. Assisted in the development of study protocols and research tools.

EDUCATIONAL BACKGROUND

Doctor of Philosophy in Epidemiology, *expected December 2007*

UNIVERSITY OF PITTSBURGH, Pittsburgh, Pennsylvania

Master of Public Health, *expected December 2007*

UNIVERSITY OF PITTSBURGH, Pittsburgh, Pennsylvania

Bachelor of Science in Human Nutrition, *1998-2002*

WEST VIRGINIA UNIVERSITY, Morgantown, West Virginia

Bachelor of Science in Animal Science, *1998-2002*

WEST VIRGINIA UNIVERSITY, Morgantown, West Virginia

TRAINING AND CERTIFICATION

Certificate in Global Health, *expected April 2008*

UNIVERSITY OF PITTSBURGH, Pittsburgh, Pennsylvania

Ovarian Cancer Speaker's Bureau Training, *2004*

NATIONAL OVARIAN CANCER COALITION (NOCC), Pittsburgh, Pennsylvania

SCHOLARSHIPS

Public Health Dean's Scholarship, University of Pittsburgh, 2004

Mountaineer Scholarship, West Virginia University, 1998-2002

Berry Scholarship, West Virginia University, 1998

GRANTS

Principal Investigator "The 6:3 PUFA ratio and intermediate markers of breast cancer." Funded by the U.S Army Medical Research and Material Command (USAMRMC), Department of Defense Breast Cancer Research Program, Predoctoral Award. April 2006- April 2009. $89,992

HONORS AND AWARDS

American Association for Cancer Research (AACR) Scholar-in Training Award, 2007

2nd place best overall presentation, Graduate School of Public Health's Deans Day, 2007

Student Travel Award, Graduate School of Public Health, 2007

Graduated with Distinction in Human Nutrition Major, 2002

Graduated *cum laude*, 2002

Gamma Beta Phi Society, 2002

Phi Upsilon Omicron Honor Society, 2001

Golden Key National Honor Society, 1999

National Society of Collegiate Scholars, 1999

MANUSCRIPTS

Burke LE, **Hudson AG**, Styn MA, Warziski M, Music E, Ulci OE, Sereika SM. Effects of a vegetarian diet and treatment preference on biological and dietary variables in overweight and obese adults: a randomized trial.(In press at American Journal of Clinical Nutrition)

Burke LE, Styn MA, Warziski M, Music E, **Hudson AG**, Sereika SM. The effects of diet preference in combination with standard diet or lacto-ovo-vegetarian diet on weight loss: a randomized controlled trial.(Under review at International Journal of Obesity)

ABSTRACTS

Hudson A, Gierach G, Modugno F, Simpson J, Vogel V, Wilson J, Evans R, Weissfeld J. Anti-inflammatory drug use and serum estradiol in postmenopausal women. Society for Epidemiological Research Annual Meeting 2007. Boston, MA. June 19-22, 2007.

Gierach G, Modugno F, Cauley J, Weissfeld J, Wilson J, Simpson J, Allen G, Romkes M, **Hudson A**, Vogel V. A non-synonymous single nucleotide polymorphism in the TNF-α receptor II gene and mammographic density. Society for Epidemiological Research Annual Meeting 2007. Boston, MA. June 19-22, 2007.

Hudson A, Weissfeld J, Modugno F, Wilson J, Evans R, Gierach G, Simpson J, Vogel V. The 6:3 PUFA ratio and serum estradiol in postmenopausal women. American Association for Cancer Research Annual Meeting 2007. Los Angeles, CA. April 14-18, 2007.

Ewing L, **Hudson A**, Warziski M, Styn MA, Elci OU, Sereika SM, Burke LE. Differences in baseline binge eating scale scores predict participants' completion and return for follow-up in a weight loss trial. Society of Behavioral Medicine's 28th Annual Meeting. Washington, DC. March 21-24, 2007.

PROFESSIONAL SERVICES

Reviewer, *American Journal of Epidemiology,* 2007

PROFESSIONAL MEMBERSHIPS

American Association for Cancer Research (AACR), 2007

American Association for the Advancement of Science (AAAS), 2007

Pennsylvania Public Health Association (PPhA), 2003-Present

SERVICE EXPERIENCE

Volunteer Administrative Support, Walk for ALS, Pittsburgh PA, 2004

Volunteer Administrative Support, Clinical Research Services, University of Pittsburgh Hillman Cancer Center, 2003-2004

Volunteer Note-taker, Rural Public Health Research Agenda Meeting, Center for Rural Health Practice, University of Pittsburgh, 2003